REFINING iPSC-BASED 3D NEURAL CELL MODELS AND CHARACTERIZATION TOOLS TO ADRESS BRAIN MICROENVIRONMENT RELATED DISEASES

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Brain microenvironment plays important roles in neurodevelopment and pathology and can affect therapy efficacy. Neural cell culture typically relies on the use of heterologous matrices that poorly resemble brain extracellular matrix (ECM) or reflect its pathological features. We have shown that perfusion bioreactor-based 3D differentiation of iPSC-derived human neural stem cells (hiPSC-NSC) sustains the concomitant differentiation of the three neural lineages (neurospheroids). If this neurospheroid culture strategy also allows deposition of native neural ECM it would be possible to (i) mimic cellular and microenvironment remodeling during neural differentiation, without the confounding effects of exogenous matrices and (ii) recapitulate pathological phenotypic features of diseases in which homotypic/ heterotypic cell-cell interactions and ECM are relevant. To characterize the neural extracellular space we employed quantitative transcriptomic (NGS) and proteome (SWATH-MS) analysis. Neurogenic developmental pathways were recapitulated in neurospheroids, with significant changes in cell membrane and ECM composition along differentiation; a significant enrichment in structural proteoglycans, typical of brain ECM, a downregulation of basement membrane proteins constituents and a higher expression of synaptic and ion transport machinery were observed. Neurospheroids were generated using hiPSC-NSC derived from Mucopolysaccharidosis type VII (MPS VII) patients. MPS VII is a rare neuronopathic lysosomal storage disease caused by deficient β-glucuronidase (β-gluc) activity, leading to glycosaminoglycan (GAGs) accumulation in the brain. The main MPS VII molecular hallmarks were recapitulated, e.g. accumulation og GAGs. By combining the neurospheroid culture with a 3D neuronal connectivity assay based on calcium imaging analysis we refined a new analytical strategy to characterize neuronal connectivity defects in a more predictive setting. We showed that MPS VII neurospheroids presented reduced neuronal activity and disturbances in network functionality, with alterations in connectivity and synchronization. These data provide insights into the interplay between reduced β-gluc activity, GAGs accumulation, alterations in neuronal network and its impact on MPS VII-associated cognitive defects Applying the characterization tools refined in this work to cope with 3D neurospheroid cultures, namely the neuronal connectivity assay, we provide a new platform to unveil the cellular processes responsible for brain dysfunction in neurological disorders and to test and optimize new therapies.

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